ATTACHMENT I--FINAL RISK ASSESSMENT OF *CLOSTRIDIUM ACETOBUTYLICUM*

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I. INTRODUCTION

Clostridium acetobutylicum is an anaerobic, saccharolytic and proteolytic bacterium that has been isolated from a number of environments. The bacterium produces endospores which allows for long-term survival in the environment even in the presence of oxygen. It exists in the biologically inactive spore stage in soils except when vegetative growth is stimulated by anaerobiosis and other favorable growth conditions. Although other members of the genus produce some of the most lethal neurotoxins known, *C. acetobutylicum* is considered a benign microorganism. Throughout its long history of use for production of acetone and butanol, there have been no reports of adverse effects to human health or the environment. It is not pathogenic or toxigenic to humans, animals, or plants. The potential risks associated with the use of this bacterium in fermentation facilities are low.

History of Commercial Use and Products Subject to TSCA Jurisdiction

C. acetobutylicum has a long history of safe use in the industrial production of acetone and butanol in fermentation systems using maize mash, molasses, or other feedstocks. Jones and Woods (1986) have thoroughly documented its history of use for solvent production. Between 1912 and 1914, Weizmann isolated a number of cultures capable of producing acetone and butanol, the most efficient of which was designated BY and later named *C. acetobutylicum*. With the outbreak of World War I and the need for acetone in munitions manufacturing, a plant was erected in England in 1916, and several existing distilleries were recruited for acetone production using maize as a substrate. However, the German blockade affected the supply of grain which made it necessary to erect another plant where grain was more readily available. An existing distillery in Canada was recruited for acetone production using the Weizmann process and strain. This plant remained in operation until the end of the war. When the United States entered the war in 1917, several plants were established in Indiana. These plants were also closed at the end of the war when the need for acetone diminished. However, the rapidly expanding automobile industry after the war created a market for butanol as a solvent in nitrocellulose lacquers for car finishes. The plants in Indiana were reopened and additional plants were built. In 1936, the patent on the Weizmann strain

expired, and new plants were built in Baltimore, MD, Philadelphia, PA, and in Puerto Rico. After 1936, acetone-butanol fermentation plants were also constructed in Japan, India, Australia, and South Africa. It is unknown whether these additional plants utilized *C. acetobutylicum* or other solventogenic clostridia. In the 1950's and 1960's, the lower costs associated with chemically-produced solvents and shortages of feedstocks led to the closing of microbial production plants in the United States and in other countries. The plant in South Africa which presumably utilized a strain of *C. acetobutylicum*, P262, remained in operation until the early 1980's (Jones and Woods, 1986). It is unknown whether plants in other countries are still in production. In recent years, production of acetone and butanol by *C. acetobutylicum* in the utilization of agricultural and domestic wastes such as whey, wood shavings, bagasse, and rice straw has also been investigated (McNeil and Kristiansen, 1986).

The solvents produced by *C. acetobutylicum*, predominately acetic acid, butyric acid, acetone, and butanol are industrial chemicals that may be subject to TSCA. The bacterium also produces small amounts of lactic acid, ethanol, and succinate that could also be subject to TSCA.

II. IDENTIFICATION AND TAXONOMY

A. Overview

C. acetobutylicum is a saccharolytic and proteolytic bacterium that has been isolated from soils, lake sediments, well water, clam gut, and from bovine, canine, and human feces (Cato et al., 1986). Although Gram positive at early stages of growth, it is pleomorphic at later stages. This bacterium is rod-shaped, motile by peritrichous flagella, and produces subterminal endospores. *C. acetobutylicum* is an obligate anaerobe, and therefore, will not grow in the presence of oxygen. However, vegetative cells may survive oxygen exposure for several hours (Gottschalk et al., 1981). The resistant endospores produced by this bacterium enable it to survive in the environment for many years, even in the presence of oxygen. *C. acetobutylicum* is capable of fixing atmospheric nitrogen, and some strains produce inducible carboxymethyl cellulase and cellobiase enzymes (Cato et al., 1986).

B. Taxonomy and Characterization

The genus *Clostridium*, which was first described in 1880, consists of a large number of species with a wide range in biochemical and physiological traits (Cato et al., 1986). There are only four criteria that need to be met for an isolate to be assigned to the genus *Clostridium*. These are: (1) the ability

to form endospores, (2) anaerobic energy metabolism, (3) the inability for dissimilatory sulfate reduction, and (4) possession of a Gram positive cell wall (that may react Gram negative) (Andreesen et al., 1989). It has been suggested that the current genus, which is not well-defined, be subdivided into six groups because of its diversity (Cato and Stackebrandt, 1989). According to Gottschalk et al. (1981), there is no standard classification system for the genus *Clostridium*, and in many cases with the nonpathogenic species, the taxonomic descriptions are incomplete and are based on single strains only. Historically, species in the genus were defined more on functional grounds, i.e., their role in the environment or ability to cause human infection, rather than on a solid phenotypic or genotypic basis. In recent years, the species in the genus have been better delineated. *C. acetobutylicum* is listed in Bergey's Manual of Systematic Bacteriology as a distinct species (Cato et al., 1986). Although the number of simple phenotypic biochemical tests required to differentiate this species from others is quite large and not easily performed, there are several basic reactions which will separate *C. acetobutylicum* from the human pathogenic species. The group of histolytic clostridia, including *C. perfringens* and *C. histolyticum*, and the major toxin producers, *C. botulinum* and *C. tetani*, all produce extracellular proteinase with gelatin as a substrate while *C. acetobutylicum* does not. Therefore, there is little chance of confusing *C. acetobutylicum* with the most pathogenic species of the genus (Edberg, 1991).

In regards to other nonpathogenic species, *C. acetobutylicum* supposedly can be differentiated from *C. baratii*, as the latter produces large amounts of lactic acid in addition to acetic and butyric acids. However, *C. acetobutylicum* does produce some lactic acid. Likewise, *C. butyricum* produces large amounts of formic acid in addition to acetic and butyric acids, whereas *C. acetobutylicum* does not produce formic acid (Edberg, 1991). However, McNeil and Kristiansen (1986) stated that the taxonomy of solventogenic clostridia based on end product analysis is an unreliable means of species classification because solvent production is a highly variable trait (George et al., 1983), and solvent production can change or be manipulated depending on the composition of the fermentation medium.

According to Johnston and Goldfine (1983), related clostridial species could be separated by thin layer chromatography based on their principal phospholipids and the degree of saturation of those lipids. It appears, therefore, that it may be possible to differentiate closely related species based on fatty acid and lipid analysis. However, these methods are not routinely available nor have these methods been applied to industrial strains of *C. acetobutylicum* (Edberg, 1991).

Even within the species of *C. acetobutylicum*, the taxonomy is somewhat confused. The five most commonly used strains of *C. acetobutylicum* strains differ widely with respect to their growth and their physiological, biochemical, and fermentative characteristics (Woolley and Morris, 1990). It has been suggested that there are at least two groups within the species (Woolley and Morris, 1990). There is apparently little DNA homology between the two proposed groups, the first of which would consist of strains NCIB 8052 and P262 (a widely used industrial strain) and the other group which would contain the type strain, ATCC 824, and DSM 1731 (Woolley, 1988). Note that in the 1989 ATCC Catalogue of Bacteria and Phages (Gherna, 1989) and in an earlier publication (Gottschal and Morris, 1982), ATCC 824 and NCIB 8052 were stated as being identical strains, but in the above proposed classification, they would fall into different DNA homology groups. Strain N1-4, formerly known as *C. saccharoperbutylacetonicum* but presently designated as *C. acetobutylicum*, apparently does not fit well into either group. Not all strains of *C. acetobutylicum* have been compared for DNA homology, nor have phylogenetic studies using rRNA sequencing been done.

C. Related Species of Concern

The taxonomic uncertainty associated with *C. acetobutylicum* is of concern in that closely related nontoxigenic clostridia have acquired the ability to produce toxins. Toxigenic members of the genus produce some of the most lethal neurotoxins known, tetanus and botulinum toxins (Shone and Hambleton, 1989). Typically, *C. botulinum* is the causal agent of both food-borne and infant botulism. In food-borne botulism, the pre-existing toxin is ingested and absorbed. In infant botulism, there is colonization and multiplication of the organism in the intestinal tract of the infant, where the toxin is then produced and absorbed (Hall et al., 1985). Recently, there have appeared three reports of infant botulism caused by botulinum toxin types E and F, that were produced not by *C. botulinum* but by nontoxigenic clostridia. In these three cases, typically nonpathogenic, nontoxigenic organisms identified as *C. baratii* and *C. butyricum* were shown to be capable of producing the botulism type F and type E toxins, respectively, (Hall et al., 1985; McCroskey et al., 1986; Aureli et al., 1986). However, as previously stated, it is possible to taxonomically distinguish *C. acetobutylicum* not only from the toxigenic species such as *C. botulinum* and *C. tetani*, but also from other closely related solventogenic species such as *C. butyricum* and *C. baratii*.

III. HAZARD ASSESSMENT

A. Human Health Hazards

There are several reports in the literature that suggest that *C. acetobutylicum* is, at least intermittently, part of the normal flora of the human colon, although it does not appear to be a major component (McNeil and Kristiansen, 1986; Jones and Woods, 1986; Awang et al., 1988). Except for its isolation from human feces, *C. acetobutylicum* has not been associated with humans. There are no reports in the literature suggesting that *C. acetobutylicum* has the ability to produce mammalian toxins, nor does it produce enzymes known to be associated with virulence. It apparently does not produce any extracellular or intracellular materials that would be toxic to humans. A search of a list of toxic substances produced by bacteria failed to reveal this species (Gill, 1986). Since *C. acetobutylicum* apparently produces no virulence factors, one would expect an extraordinarily large number of microorganisms to be required to cause even a superficial infection (Edberg, 1991).

The major threat to human health would be the acquisition of toxin production genes from botulism toxin-producing members of the genus. As mentioned previously, closely related species, *C. baratii* and *C. butyricum*, have been shown to acquire the botulism toxin production genes from clostridial pathogens. An isolate of *C. baratii* was repeatedly isolated from the stool of an infant suffering from infant botulism (Hall et al., 1985). McCroskey et al. (1986) and Aureli et al. (1986) described the isolation of *C. butyricum* from infants with type E botulism. The Center for Disease Control further analyzed these strains of *C. baratii* and *C. butyricum* and confirmed that they did, in fact, produce type F and type E neurotoxins that were similar to, or indistinguishable from, those produced by *C. botulinum*. Gimenez and Sugiyama (1988) studied the type E toxin produced by *C. butyricum* and found it was very similar to that produced by *C. botulinum*, and that the LD_{50} for mice and the immunological cross-reactivity were extremely similar. The identification of both these organisms in question was confirmed by DNA reassociation studies with the type strains from these species (Suen et al., 1988). Therefore, it appears that closely related clostridial species can acquire the ability to produce botulinum toxins. Presumably, these toxins are coded for by extrachromosomal elements such as plasmids. Although there is no evidence in the literature that *C. acetobutylicum* can acquire genetic material from other bacteria, few studies on gene transfer have been conducted with this microorganism. It is theoretically possible, therefore, that *C. acetobutylicum*, like *C. baratii* and *C. butyricum*, could acquire the ability to produce toxins from toxigenic clostridia.

Infant botulism, however, is a rare disease. The Center for Disease Control estimates that there are approximately 100 confirmed cases of infant botulism per year. It seems remote that the events necessary for an infant to acquire botulism from *C. acetobutylicum* would occur in an industrial setting. First, the strain would have to acquire the genetic basis to elaborate a

botulinum toxin. Second, an infant present in the fermentation facility would have to ingest a large number of spores with the ability of the spores to form viable vegetative cells in the immature pediatric gastrointestinal system. Good industrial practices would certainly preclude the presence of infants in fermentation facilities. Consequently, the concern for infant botulism from the industrial use of this species is remote (Edberg, 1991).

There is, however, also a condition known as wound botulism following trauma, whereby a large number of spores are inoculated into an open wound. Typically, this disease is caused by *Clostridium botulinum*. If *C. acetobutylicum* strains acquired the ability to produce botulism toxin as did the other nonpathogenic species mentioned above, there is a possibility that high concentrations of vegetative cells or spores could accidentally be inoculated into a wound. However, good worker hygiene, including use of protective clothing, should mitigate this concern.

The likelihood of toxin acquisition by an industrial strain with a history of safe use in an industrial setting seems remote. The only concern is that new strains or environmental isolates of *C. acetobutylicum* may have had contact with clostridial pathogens and acquired toxin-production genes. It may be prudent for the manufacturer to screen culture supernatants at late log phase of growth for the production of botulism toxins (Edberg, 1991). Since it is highly unlikely that *C. acetobutylicum* would acquire botulinum toxin genes, the overall human health risk of *C. acetobutylicum* is minimal (Edberg, 1991).

B. Environmental Hazards

1. Hazards to Animals

There are no reports in the literature suggesting that *C. acetobutylicum* is an animal pathogen (McClung, 1991), and it is not listed as such in a review of animal pathogens by Hill (1981). As mentioned above, *C. acetobutylicum* has not been shown to produce any toxins, enzymes, or virulence factors typically associated with mammalian toxicity or pathogenicity (Edberg, 1991).

The only remote hazard to animals would be the acquisition of botulism toxin genes as shown for closely related solventogenic clostridia. Botulism toxins have been shown to be toxic to mice (Cato et al., 1986), and the supernatant culture fluid from strains of type E botulism toxin-producing *C. botulinum* were toxic to gallinaceous birds (pheasants, turkeys, grouse, and domestic fowl) (Gross and Smith, 1971).

2. Hazards to Plants

There are no reports in the literature indicating that *C. acetobutylicum* has any adverse effects on plants. No members of the genus *Clostridium* are listed as plant pathogens according to the Federal Plant Pest Act (7 CFR 330, et seq.)

3. Hazards to Other Microorganisms

C. acetobutylicum (P262) produces a bacteriocin (Barber et al., 1979) near the end of the exponential growth stage. Bacteriocins are usually thought to have bactericidal action against the same species or other clostridial species. Barber et al. (1979) reported that this bacteriocin had inhibitory effects against members of the same species and against one other clostridial species, *C. felsineum*. The bacteriocin from *C. acetobutylicum* (P262) was not inhibitory to *Achromobacter*, *Escherichia coli, Serratia marcescens*, *Salmonella typhimurium*, or *Bacteroides fragilis* (Barber et al., 1979). Soucaille and Goma (1986) reported that a bacteriocin obtained from *C. acetobutylicum* strain ATCC 824 was similar to that obtained from strain P262 (Barber et al., 1979). The bacteriocin from ATCC 824 was not inhibitory to *Corynebacterium glutamicum, E. coli, Proteus mirabilis, Aerobacter aerogenes*, or *Zymomonas mobilis*, but was inhibitory to *C. butyricum* and members of the family Bacillaceae including *Bacillus subtilis* and *B. megaterium* (Soucaille and Goma, 1986). The production of bacteriocins in the environment by *C. acetobutylicum* would not be likely to be of concern. Although *C. acetobutylicum* is expected to survive in the environment, it will exist predominantly as spores rather than as vegetative cells since it is obligately anaerobic. Even if bacteriocins are released into the environment with spent fermentation wastes, there is still little environmental concern due to the high numbers of bacilli typically found in soils. The levels of members of the genus *Bacillus* are thought to range between 10^6 - 10^7 per gram of soil (Alexander, 1977). In addition, *C. acetobutylicum* is widespread in the environment. Under some conditions, population levels of 10^6 clostridia per gram of soil have been found (Alexander, 1977).

4. Hazards Posed to Other Processes

Although *C. acetobutylicum* fixes atmospheric nitrogen, the amount of nitrogen fixed by this organism in the environment would probably be negligible, as it is expected to survive predominately as spores. In addition, the amount of nitrogen fixed by nonsymbiotic microorganisms is relatively small compared to symbiotic associations. The numbers of N_2-fixing clostridia in arable soils range from 10^2 to 10^6 per gram of soil, of which *C. acetobutylicum* is thought to be one of three prominent species

(Alexander, 1977). However, in the environment, nitrogen fixation rates would not be expected to be appreciable, as the efficiency of nonsymbiotic nitrogen fixation is low and energy sources are scarce.

Some strains of *C. acetobutylicum* produce cellulases which enable the fermentation of some feedstocks, but this does not appear to be a potential environmental hazard since this organism already exists in the environment (Alexander, 1977), and survival would most likely be in the spore stage.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

C. acetobutylicum is considered a Class 1 Containment Agent under the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (U.S. Department of Health and Human Services, 1986).

No data were available for assessing the release and survival specifically for fermentation facilities using *C. acetobutylicum* because commercial anaerobic fermentation processes are currently at the research stage of development. The releases from an anaerobic process are expected to be no higher than those for an aerobic system (Reilly, 1991; Macek, 1992). Therefore, for purposes of this assessment, the potential worker exposures and routine releases to the environment from large-scale, conventional aerobic fermentation processes estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms will be used (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, i.e., near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in

air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

No studies exist in the literature on the survival of *C. acetobutylicum* specifically, although it has been isolated from many environments (Cato et al., 1986). This bacterium is an obligate anaerobe and growth is inhibited in the presence of oxygen. However, vegetative cells can survive exposure to oxygen for several hours (Gottschalk et al., 1981). Typically, *C. acetobutylicum* exists as endospores which are quite resistant to adverse environmental conditions and can survive for many years. Its widespread presence in nature, its ability to colonize anaerobic environments, and the resistance of its endospores indicate that released microorganisms are likely to survive outside of containment (Versar Inc., 1992).

2. Releases

Estimates of the number of *C. acetobutylicum* organisms released during production are tabulated in Table 1 (Reilly, 1991). The uncontrolled/untreated scenario assumes no control features for the fermentor off-gases, and no inactivation of the fermentation broth for the liquid and solid waste releases. The containment criteria required for the full exemption scenario assume the use of features or equipment that minimize the number of viable cells in the fermentor off-gases. They also assume inactivation procedures resulting in a validated 6-log reduction of the number of viable microorganisms in the liquid and solid wastes relative to the maximum cell density of the fermentation broth.

TABLE 1. Estimated Number of Viable *C. acetobutylicum* Organisms Released During Production

Release Media	Uncontrolled/ Untreated (cfu/day)	Full Exemption (cfu/day)	Release (days/yr)
Air Vents	$2x10^{8}$ - $1x10^{11}$	$<2x10^{8}$ - $1x10^{11}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{16}$	$7x10^{10}$	90
Soil/Landfill	$7x10^{18}$	$7x10^{12}$	90

Source: Reilly, 1991

These are "worst-case" estimates which assume that the maximum cell density in the fermentation broth for bacteria is 10^{11} cfu/ml, with a fermentor size of 70,000 liters, and the separation efficiency for the rotary drum filter is 99 percent.

3. Air

Specific data which indicate the survivability of *C. acetobutylicum* in the atmosphere after release are currently unavailable. Survival of vegetative cells during aerosolization is typically limited due to stresses such as shear forces, desiccation, temperature, and UV light exposure. In addition, vegetative cells of *C. acetobutylicum* cannot survive in the presence of oxygen except for brief periods. However, its ability to produce endospores suggests that this organism may survive after release. As with naturally-occurring strains, human exposure may occur via inhalation as the organisms are dispersed in the atmosphere attached to dust particles, or lofted through mechanical or air disturbance.

Air releases from fermentor off-gas could potentially result in nonoccupational inhalation exposures due to point source releases. To estimate exposures from this source, the sector averaging form of the Gaussian algorithm described in Turner (1970) was used. For purposes of this assessment, a release height of 3 meters and downward contact at a distance of 100 meters were assumed. Assuming that there is no removal of organisms by controls/equipment for off-gases, potential human inhalation dose rates are estimated to range from 3.0×10^{3} to 1.5×10^{6} cfu/year for the uncontrolled/untreated scenario and less than that for systems with full exemptions. It should be noted that these estimates represent hypothetical exposures under reasonable worst case conditions (Versar, 1992).

4. Water

The concentrations of *C. acetobutylicum* in surface water were estimated using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/l). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers (IFD) database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers facilities that have a NPDES permit to discharge to surface water). Tenth percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year period. The use of this methodology to estimate concentrations of *C. acetobutylicum* in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process. Estimated concentrations of *C. acetobutylicum* in surface water for the uncontrolled/ untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. *C. acetobutylicum* Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	7Q10	Mean	7Q10
Uncontrolled/Untreated				
10th Percentile	156	5.60	4.5×10^{8}	1.25×10^{10}
50th Percentile	768	68.13	9.11×10^{7}	1.03×10^{9}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{2}	1.25×10^{4}
50th Percentile	768	68.13	9.11×10^{1}	1.03×10^{3}

*MLD = million liters per day
Source: Versar, 1992

5. Soil

Since soil is a natural habitat for *C. acetobutylicum*, long-term survival in soil may be expected to occur, particularly under anaerobic conditions. The resistant endospores formed could promote survival for years, even under aerobic conditions. Human exposures via dermal and ingestion routes, and environmental exposures (i.e., to terrestrial, avian, and aquatic organisms via runoff) may occur at the discharge site because of the potential establishment of *C. acetobutylicum* within the soil.

6. Summary

Although direct monitoring data are unavailable, worst case estimates do not suggest high levels of exposure of *C. acetobutylicum* to either workers or the public resulting from normal fermentation operations.

V. INTEGRATION OF RISK

A. Discussion

C. acetobutylicum is a common soil proteolytic and saccharolytic bacterium which is widespread in nature. Population levels of clostridia, of which *C. acetobutylicum* is thought to predominate, range from 10^2 to 10^6 per gram of soil. This bacterium is obligately anaerobic implying growth only under reducing conditions, although the vegetative cells have been shown to survive for several hours with oxygen exposure. It exists predominately in the environment as endospores which are quite resistant to adverse environmental conditions such as heat, desiccation, low nutrient status, and aerobic conditions. Releases from the fermentation facility of vegetative cells into anaerobic environments, or of spores into any environment, would, most likely, result in survival of the bacterium.

There are no reports of ecological or human health hazards caused by *C. acetobutylicum*. This bacterium is not thought to be a pathogen of either plants or animals. It does not produce any toxins, enzymes, or virulence factors normally associated with mammalian toxicity. Although it can, intermittently, occupy the human intestines, it is not thought to be a major component of the normal human flora. Except for its isolation from human feces, it is not otherwise associated with humans.

The only potential risk associated with *C. acetobutylicum* is the possibility of acquiring toxin-producing genes from pathogenic clostridia. *Clostridium botulinum* and *C. tetani* produce some of the most lethal neurotoxins known. There are no reports in the literature indicating that *C. acetobutylicum* can acquire these toxin genes. However, other closely related solventogenic, nontoxigenic clostridia have acquired the ability

to produce botulism toxin types E and F. There are three cases of infant botulism which were caused by a strain of *C. baratii* and two strains of *C. butyricum*. The botulism toxins produced by these typically nontoxigenic clostridia were indistinguishable from the botulism toxins produced by *C. botulinum*. The toxin genes in *C. botulinum* are presumably coded for by extrachromosomal elements such as plasmids. It appears that the nonpathogenic *Clostridium* species acquired the toxin-producing genes from *C. botulinum*. Although there are no reports indicating that *C. acetobutylicum* can acquire toxin genes, only a limited number of gene transfer studies have been conducted with this bacterium. It is theoretically possible that *C. acetobutylicum* could acquire botulism toxin genes.

The only concern for the acquisition of botulism toxin genes lies with the use of strains or environmental isolates that may have been in contact with the toxigenic bacteria. The likelihood of acquisition of a toxin gene by an industrial strain of *C. acetobutylicum* with a history of safe use in an industrial setting is remote due to the fact that care is taken to prevent contamination of the fermentors by other microorganisms. The only cases in which the closely related nontoxigenic clostridia acquired the toxin genes involved infant botulism. The conditions needed for development of infant botulism would not occur in fermentation facilities as exposure to large numbers of spores directly by the infant is necessary. Wound botulism of industrial workers is also highly unlikely since large numbers of vegetative cells or spores need to be inoculated into an open wound. Under good industrial practices, the fermentation workers would be expected to be wearing protective clothing and participating in good industrial hygiene which would allay this risk of infection given an accidental spill. All of these scenarios for botulism toxicity are dependent on the acquisition of the toxin gene by the organism which is theoretically possible, but has never been shown.

Industrial strains of *C. acetobutylicum* have a long history of safe use in the production of acetone and butanol with no incidents of adverse effects to human health or the environment. This organism is considered benign, and the only associated potential hazard with it is the theoretical possibility of acquisition of botulism toxin genes from toxigenic clostridia. Toxin acquisition has never been reported for this particular species, and the likelihood is remote, especially in an industrial setting where efforts are taken to minimize contaminants of the fermentation. If released into the environment, this bacterium is expected to survive, predominately as resistant spores except under anaerobic conditions. However, naturally-occurring *C. acetobutylicum* strains are widespread in the environment, and the limited exposure resulting from the use of *C. acetobutylicum* in fermentation facilities will not affect the population size of this species in the environment. Both the

hazard and the exposure associated with the use of *C. acetobutylicum* are low. Therefore, the risks to human health and the environment associated with the use of this microorganism are low.

B. Recommendations

Clostridium acetobutylicum is recommended for the tiered exemption.

VI. REFERENCES

7 CFR 330, et seq., as amended.

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